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TITLE: Integration of Pathologic Findings with Clinical-Radiologic Tumor Measurements to Quantify Response to Neoadjuvant Chemotherapy

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14. ABSTRACT <p>The aim of the project is to develop and test a new method to quantify the proportion (percent) of cancer that is residual after neoadjuvant chemotherapy using standard radiologic and/or clinical measures of tumor size that are integrated with pathologic information about the amount of cancer within each tumor. In years 1 and 2 we combined our measure of cancer cellularity with the radiological tumor measurements with the gross and microscopic pathologic changes in the breast and axillary lymph nodes after chemotherapy to determine a measure of relative breast cancer response. This Residual Cancer Index (RCI) closely correlated with T-stage and greater proportion of residual cancer (poor response) after paclitaxel, 5-FU, doxorubicin, and cyclophosphamide (T/FAC) was significantly related to low proliferation, bcl-2 overexpression, and tau overexpression. By the end of year 3 (June 2005) we had enlarged the evaluation to 103 patients who received T/FAC chemotherapy (6 months) and 61 patients who received FAC alone (3 months neoadjuvant then 3 months adjuvant), conducted an interim evaluation of distant relapse-free survival (DRFS) with respective median follow-up of 48 and 90 months, and compared the amount of residual cancer burden (RCB) at the completion of neoadjuvant treatment with the proportional reduction of cancer represented by RCI. We found that in both cohorts the new measures of response were significantly prognostic and outperformed the existing classification of response as pathologic complete response (pCR) versus residual disease (RD). There was no prognostic difference between RCI and RCB. Therefore, we have selected RCB to evaluate in a larger cohort of patients who received neoadjuvant T/FAC (n=243) or FAC or T alone (n=189) and for whom the pathologic material after treatment is available for review. We requested a one-year extension to complete this and extend the follow-up period.</p>					
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INTRODUCTION:

A more accurate way to measure breast cancer response to treatment would improve the rate of yield of information from clinical trials of neoadjuvant chemotherapy. It would also provide a more useful standard with which to compare the relevance of pathologic findings in residual cancer and with which to test those molecular biomarkers that show promise to predict response to treatment. We are developing and testing a method to quantify tumor response, using a combination of clinical, radiologic, and pathologic information that is applicable to most clinical practices. We are comparing the molecular evidence of cell survival and proliferative activity in the residual cancer cells and pathologic changes in the residual carcinoma from neoadjuvant chemotherapy as they relate to the amount of tumor response.

BODY:

Task 1. To determine the best measurement of tumor size before & after treatment (Months 1 - 24)

- a. Review of mammography and ultrasound imaging studies from before and after treatment, estimate average of 10 cases per month. (Months 1 - 24)*
- b. Two radiologists to independently make measurements and document the preferred imaging modality for each tumor. (Months 1 - 24)*
- c. Obtain the clinical tumor measurements and the categorical assessments of tumor response from the clinical trial database. (Months 1 - 6)*
- d. Pathology review of slides, reports, and specimen radiographs to document residual tumor size and other histopathologic findings for subsequent tasks. (Months 1 - 24)*
- e. Complete the statistical analyses. (Months 24 - 25)*

The Department of Defense approved the IRB for human subjects research on December 22, 2002. In year one we have identified a cohort of 108 patients who received neoadjuvant chemotherapy for breast cancer and reviewed their pathology materials (see task 2). Pathological data included: tumor size, % invasive cancer, % in situ cancer, % cancer cellularity within the tumor, and cytomorphologic changes within residual cancer cells. In year two we completed our analysis of the pathological changes in % cancer cellularity before and after neoadjuvant chemotherapy, compared this to the clinical response and pathologic T-stage after treatment. We presented the findings as a poster at the San Antonio Breast cancer Symposium in December, and published these results as a paper in *CANCER* in March, 2004. In year two we completed the radiological review of all radiological materials from 85 of these patients (the review of remaining patients' material is ongoing) and combined these results with the pathological data to determine an index score for the proportion of residual cancer burden after chemotherapy relative to the cancer burden before treatment began.

In year 3 we completed the review of radiologic tumor measurements in 160 patients who received T/FAC and used this to collect radiologic measurements (from reports) in an additional 61 patients who received FAC. Pathologic evaluation was completed for those cases.

Task 1 is now complete.

Task 2. Calculation of percent residual cancer volume (Months 1 - 27)

- a. *Immunohistochemical staining of tumor sections for cytokeratins. (Months 1 - 24)*
- b. *Image analysis to calculate percent cancer cellularity by area. (Months 3 - 24)*
- c. *Calculation of tumor volume using the best measure of tumor size - see task 1. (Months 24 - 26)*
- d. *Calculation of percent residual cancer volume and statistical analyses. (Months 25 - 27)*

Task 2 was completed in years 1 and 2. The residual cancer index (RCI) represents the proportion of cancer that is residual after neoadjuvant chemotherapy and was calculated using the following formula:

$$RCI = [(Residual\ Pathological\ tumor\ area \times Proportion\ invasive\ cancer) + (\# \text{ Positive lymph nodes} \times Diameter\ largest\ metastasis)] / (Pre-treatment\ Radiological\ tumor\ area \times Proportion\ invasive\ cancer)$$

In year 3 we calculated the RCI of 160 patients who received T/FAC and an additional 61 patients who received FAC. We also evaluated the residual cancer burden after treatment, as follows:

$$RCB = [(Residual\ Pathological\ tumor\ area \times Proportion\ invasive\ cancer) + (\# \text{ Positive lymph nodes} \times Diameter\ largest\ metastasis)]$$

Task 2 is now complete.

Task 3. To assess the pathology of residual cancers and correlate these with tumor response. (Months 12 - 30)

- a. *Immunohistochemical staining of residual tumor sections for Ki-67/MIB-1, HIF-1a, bcl-2, bcl-XL, and NF-k β . (Months 12 - 20)*
- b. *TUNEL assay for apoptosis in residual tumor sections. (Months 20 - 24)*
- c. *Microscopic interpretation of immunohistochemistry and TUNEL staining. (Months 20 - 28)*
- d. *Complete the statistical analyses with tumor response. (Months 28 - 30)*

Task 3 is complete. The following biomarker assays were performed on tissue sections from the clinical trial participants: ER, PR, HER2, bcl-2, bcl-6, Ki-67, NF-k β , p53, tau, and beclin-1. The following biomarkers were not performed on the clinical trial samples because of technical or interpretative failure to convince us they would provide useful information: TUNEL and HIF-1a. Bcl-XL was performed on the trial samples but failed to demonstrate reliable results and was not included in the analyses. Our analysis of the immunohistochemical staining of residual cancer cells is presented for the available tissues from these patients. Immunohistochemistry results were dichotomized as follows: Ki-67 $\geq 15\%$ of nuclei defined as positive, bcl-2 cytoplasmic staining intensity $\geq 2+$ (range 0 – 3) defined as positive, any bcl-6 nuclear staining defined as positive, any NF kappa B nuclear staining defined as positive, p53 $\geq 5\%$ of nuclei defined as positive, any survivin staining defined as positive, tau cytoplasmic staining intensity $\geq 2+$ (range 0 – 3) defined as positive, and beclin-1 cytoplasmic staining intensity $\geq 2+$ (range 0 – 3) defined as positive.

		Residual pT- Stage	Residual Tumor Size x Cellularity	Residual Cancer Index
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	n =	p value *	p value ^	p value ^
Ki-67	160	0.007	< 0.001	0.020
bcl-2	125	NS	NS	NS
bcl-6	121	NS	NS	NS
NFkB	123	NS	NS	NS
p53	122	NS	0.002	NS
Survivin	124	NS	NS	NS
Tau	132	0.008	NS	0.042
Beclin-1	140	NS	NS	NS

* Chi-Square LR test, ^ Mann-Whitney U test, NS is not significant ($p > 0.05$)

Although high proliferation index (Ki-67 expression $\geq 15\%$) was only identified in 16% of residual cancers, this finding was associated with more extensive residual disease. There was also a significant but weak trend towards more frequent tau expression in tumors with more extensive residual disease. Overall, there was not consistent or strong relationship between the expression of these biomarkers and residual tumor pT-Stage, residual tumor burden, or residual cancer index that could provide useful insight into the biology of residual disease.

Task 3 is now complete.

Task 4. To test selected potential biomarkers for prediction of tumor response. (Months 24 - 34)

- Immunohistochemical staining of pre-treatment tumor samples for Ki-67/MIB-1 and p53. (Months 24 - 30)*
- Retrieval of results from Her-2/neu tests from pathology reports. (Months 24 - 27)*
- Microscopic interpretation of immunohistochemical staining and histopathologic biomarkers. (Months 28 - 32)*
- Complete the statistical analyses with tumor response. (Months 32 - 34)*

Task 4 is complete. The following biomarker assays were performed on tissue sections from the clinical trial participants: ER, PR, HER2, bcl-2, Ki-67, p53, tau, and beclin-1. The following biomarkers were not performed on the clinical trial samples because of technical or interpretative failure to convince us they would provide useful information: TUNEL and HIF-1a. Bcl-XL, bcl-6, and NF- κ B were performed on the trial samples but failed to demonstrate reliable results, and were not included in the analyses. Our analysis of the immunohistochemical staining of residual cancer cells is presented for the available tissues from these patients. Immunohistochemistry results were dichotomized as follows: ER and PR $\geq 10\%$ of nuclei as positive, HER2 positive if membrane staining 3+ intensity or gene amplification, Ki-67 $\geq 15\%$ of nuclei defined as positive, bcl-2 cytoplasmic staining intensity $\geq 2+$ (range 0 – 3) defined as positive, any bcl-6 nuclear staining defined as positive, any NF kappa B nuclear staining defined as positive, p53 $\geq 5\%$ of nuclei defined as positive, any survivin staining defined as positive, tau cytoplasmic staining intensity $\geq 2+$ (range 0 – 3) defined as positive, and beclin-1 cytoplasmic staining intensity $\geq 2+$ (range 0 – 3) defined as positive.

	n =	Residual pT-Stage	Residual Tumor Size x Cellularity	Residual Cancer Index
		p value *	p value ^	p value ^
ER	139	< 0.001	< 0.001	< 0.001
PR	139	NS	NS	NS
Her2	136	NS	NS	NS
Ki-67	111	0.009	NS	0.025
bcl-2	113	0.03	0.001	0.018
p53	112	NS	NS	NS
Tau	115	< 0.001	0.02	0.001
Beclin-1	113	0.005	0.003	0.001

* Chi-Square LR test, ^ Mann-Whitney U test, NS is not significant (p>0.05)

Breast cancers with greater proliferation (Ki-67 \geq 15%) were associated with smaller residual tumor pT-Stage, less residual tumor burden, and smaller Residual Cancer Index scores. This is a meaningful result because the tumor proliferation index before treatment has been previously shown to be related to greater probability of achieving a complete pathological response, versus residual disease. Breast cancers with bcl-2 overexpression had significantly greater residual tumor pT-Stage, residual tumor burden, and Residual Cancer Index scores. This is an interesting finding because other studies have shown only borderline significance of bcl-2 overexpression to predict complete pathological response versus residual disease. Our analyses demonstrate that bcl-2 overexpression is probably more predictive of the amount of residual tumor burden and higher Residual Cancer Index scores. That makes sense when we consider the underlying hypothesis that bcl-2 overexpression would confer more resistance. It is interesting to note that overexpression of tau protein is associated with a more residual tumor and higher Residual Cancer Index score. We identified from a different study using gene expression microarray experiments from pre-treatment FNA tumor samples in a different cohort of patients receiving T/FAC chemotherapy that elevated tau gene expression was strongly predictive of residual disease, versus complete pathological response. We were able to demonstrate in these patients that the immunohistochemical overexpression of tau was related to higher Residual Cancer Index scores (more residual cancer relative to the original tumor burden). A similar finding was that beclin-1 expression was also related to more extensive residual disease and higher Residual Cancer Index scores. We also identified beclin-1 from gene array data and have confirmed this association with poor response at the immunohistochemical level. The relevance of this is furthered by our genomic and immunohistochemical studies that confirm that these 3 chemoresistance markers (bcl2 for anti-apoptosis, tau for microtubule stability, and beclin-1 for autophagy and protein reprocessing) are all strongly coexpressed with ER. In the final year of funding we will use immunohistochemistry to investigate the expression of some of these molecules with survival.

Task 5. Compilation of patient follow-up from clinical trial database and statistical analyses for disease free interval and survival. (Months 30 - 36)

In year 3 we conducted a survival analysis of a cohort of 103 patients who received sequential chemotherapy with paclitaxel then 5-FU, doxorubicin, and cyclophosphamide (T/FAC) and had median follow-up of 4 years. We also evaluated survival in a separate cohort of 61 patients who received FAC chemotherapy alone, and had median follow-up of 7 years. The pathological and radiological materials were retrieved for review to calculate the Residual Cancer Index (RCI) score and the Residual Cancer Burden (RCB) score for comparison (see progress on task 2) with time to progression (distant relapse). Given the findings of task 4, in which we demonstrate ER-related

biomarkers are associated with worse response, we tested the prognostic significance of RCI and RCB in all patients and also accounting for ER status as a covariate. The recent results of those analyses were presented at the Era of Hope Meeting, June 2005.

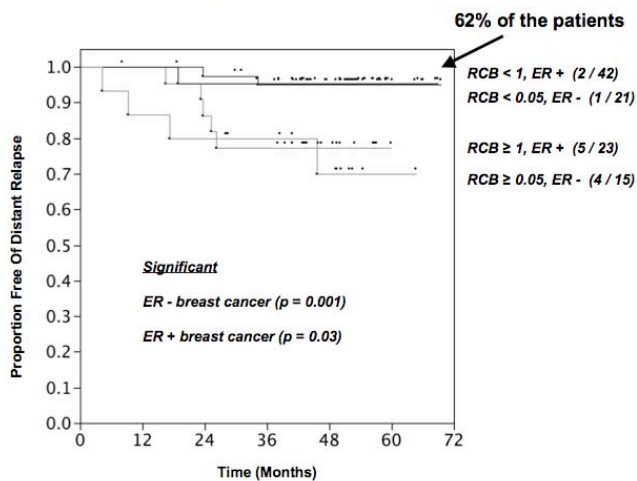
T/FAC Chemotherapy (Median Follow-up 4 Years)					
Response Parameter		N (%)	Relapse Events	Univariate DRFS P value	Multivariate with ER Status P value
pCR vs. RD	pCR	27 (26%)	2	0.4	0.3
	RD	76 (74%)	10		
Tumor Size	T0	26 (26%)	2	0.2	0.2
	T ≤ 1 cm	24 (22%)	5		
	T > 1 cm	53 (51%)	5		
RCI	Continuous (log scale)	103	12	0.2	0.09
RCB	Continuous (log scale)	103	12	0.06	0.03

The survival analyses of the T/FAC cohort after 4 years follow-up demonstrates that RCB and (to a lesser extent) RCI have stronger prognostic value than pCR or tumor size categories. This was the impetus to request the no-cost exemption in order to extend this study to a larger cohort of T/FAC patients and achieve 5 years median follow-up prior to completion of the study and reporting of results.

FAC Chemotherapy (Median Follow-up 7 Years)					
Response		N (%)	Relapse Events	Univariate DRFS P value	Multivariate with ER Status P value
pCR vs. RD	pCR	4 (7%)	0	0.15	0.2
	RD	57 (93%)	24		
Tumor Size	T0	6 (10%)	0	0.03	0.01
	T ≤ 1 cm	12 (20%)	2		
	T > 1 cm	43 (70%)	22		
RCI	Continuous (log scale)	61	24	0.008	0.006
RCB	Continuous (log scale)	61	24	0.003	0.007

The cohort who received FAC chemotherapy had longer follow-up and clearly demonstrate that RCB and RCI have stronger prognostic power than pCR or tumor size. The analyses in both cohorts suggest that evaluation of the residual cancer burden (RCB) should be sufficient. That is practically important because we usually have little difficulty obtaining the resection pathology slides to review, but have greater difficulty also obtaining the pre-treatment core biopsy slides from referring laboratories. Evaluating only RCB will allow us to complete a much larger study in the final year of this project.

Distant Relapse-Free Survival Following T/FAC Chemotherapy By RCB And ER Status



When RCB was optimized into high versus low in ER-positive and ER-negative patients, it becomes apparent that many patients in the study have an excellent survival benefit at 5 years. This is likely to be partly from chemotherapy and partly from adjuvant hormonal therapy in the ER-positive patients. Finally, in the final year we will also compare the predictive biomarkers of response (task 4) with patient survival (task 5), according to ER status.

KEY RESEARCH ACCOMPLISHMENTS:

Key research accomplishments from this study to date are:

- Demonstration that cancer cellularity within the tumor is significantly decreased by neoadjuvant chemotherapy, and is most obvious and variable in the partial response and minimal response (stable disease) categories and, similarly, in tumors staged as T1 after treatment.
- Mathematical definition of a Residual Cancer Index (RCI) score that incorporates radiological and histopathological information about the tumor before treatment and gross and histopathological information about the residual tumor and axillary nodes after the completion of neoadjuvant chemotherapy.
- Mathematical definition of a Residual Cancer Burden (RCB) score that incorporates gross and histopathological information about the residual tumor and axillary nodes after the completion of neoadjuvant chemotherapy.
- There is no obvious association between the biomarkers that we evaluated in the post-treatment residual tumor specimen and the extent of response from chemotherapy, except for the finding that a subset of 16% of the more extensive residual tumors had high proliferation, possibly a resistant subset.
- Demonstration that biomarkers that are predictive of incomplete response are linked to ER expression (bcl-2, tau, and beclin-1), and are functionally related to the pharmacologic target (microtubules), or control of cellular survival and repair of damaged cellular components. The association with poor response is stronger in the measures of the extent of residual tumor.
- Measures of the amount of tumor response (RCB and RCI) are more strongly prognostic than the current categories that are used to define response.
- There might be no prognostic difference between evaluating the relative shrinkage of tumor (RCI) compared to evaluating the extent of residual tumor (RCB).
- There is a group of patients with excellent, but incomplete, pathologic response who have similar prognosis to those who achieved pCR.

REPORTABLE OUTCOMES:

See Key Research Accomplishments above. The additional year of support (extension) will lead to the following reportable outcomes in a larger sample cohort and with mature 5-year survival data:

- Measurement of the prognostic value of RCB and RCI scores
- Measurement of the prognostic value of the biomarkers that predict tumor response, particularly in ER-positive patients
- An answer to the question of whether it is more important to measure the relative change in the tumor from treatment (RCI) or the extent of residual disease at the completion of treatment.

Abstracts:

Rajan R, Poniecka A, Smith T, Yang Y, Whitman G, Fiterman DJ, Pusztai L, Kuerer H, Hortobagyi GN, Symmans WF. Tumor cellularity of breast cancer as a variable in the pathological assessment of response following neoadjuvant chemotherapy. San Antonio Breast Cancer Symposium, December, 2003.

Symmans WF, Rajan R, Whitman G, Assad L, Adepoju L, Stephens T, Dryden M, Pusztai L, Kuerer H. A Radiologic-Pathologic Method to Quantify Response to Neoadjuvant Chemotherapy Predicts Time to Distant Recurrence. Era of Hope Conference, Department of Defense Breast Cancer Research Program, Philadelphia, PA June, 2005

Published Paper:

Rajan R*, Poniecka A*, Smith TL, Yang Y, Frye F, Pusztai L, Fiterman DJ, Gal-Gombos E, Whitman G, Rouzier R, Green M, Kuerer H, Buzdar AU, Hortobagyi GN, Symmans WF. Change in tumor cellularity of breast cancer following neoadjuvant chemotherapy as a variable in the pathological assessment of response. **Cancer** 2004;100:1365-73.

CONCLUSIONS:

1. Measurement of the residual tumor and the tumoral response is more strongly prognostic than usual categories of pathologic response, and these offer new ways to evaluate biomarkers that might predict treatment response.

REFERENCES:

Abstracts

Rajan R, Poniecka A, Smith TL, Yang Y, Frye F, Pusztai L, Fiterman DJ, Gal-Gombos E, Whitman G, Rouzier R, Green M, Kuerer H, Buzdar AU, Hortobagyi GN, **Symmans WF**. Change in tumor cellularity of breast cancer following neoadjuvant chemotherapy as a variable in the pathological assessment of response. San Antonio Breast Cancer Symposium, December 2003

Peer Reviewed Publications

Rajan R, Poniecka, Smith TL, Yang Y, Frye F, Pusztai L, Fiterman DJ, Gal-Gombos E, Whitman G, Rouzier R, Green M, Kuerer H, Buzdar AU, Hortobagyi GN, **Symmans WF**. Change in tumor cellularity of breast cancer following neoadjuvant chemotherapy as a variable in the pathological assessment of response. **Cancer** 2004;100:1365-73.

APPENDIX:

Pdf file of publication:

Rajan R, Poniecka, Smith TL, Yang Y, Frye F, Pusztai L, Fitterman DJ, Gal-Gombos E, Whitman G, Rouzier R, Green M, Kuerer H, Buzdar AU, Hortobagyi GN, **Symmans WF**. Change in tumor cellularity of breast cancer following neoadjuvant chemotherapy as a variable in the pathological assessment of response. **Cancer** 2004;100:1365-73.

Pdf file of poster from Era of Hope Meeting, June 2005:

Symmans WF, Rajan R, Whitman G, Assad L, Adepoju L, Stephens T, Dryden M, Pusztai L, Kuerer H. A Radiologic-Pathologic Method to Quantify Response to Neoadjuvant Chemotherapy Predicts Time to Distant Recurrence. Era of Hope Conference, Department of Defense Breast Cancer Research Program, Philadelphia, PA June, 2005

Change in Tumor Cellularity of Breast Carcinoma after Neoadjuvant Chemotherapy As a Variable in the Pathologic Assessment of Response

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BACKGROUND. Complete pathologic response of breast carcinoma to neoadjuvant chemotherapy is a well defined outcome that correlates with prolonged survival. Categorization of incomplete response depends on accurate measurement of residual tumor size but is complicated by the variable histopathologic changes that occur within the tumor bed. In the current study, the authors investigated the contribution of assessing tumor cellularity in the pathologic evaluation of response to chemotherapy.

METHODS. The slides from diagnostic core needle biopsy and the subsequent matched resection specimens were examined in 240 patients with breast carcinoma: 120 "treated" patients who received neoadjuvant chemotherapy and 120 "control" patients who received primary surgical management within a few weeks of diagnosis. Clinical response and residual tumor size were evaluated in 108 treated patients who completed a clinical trial with paclitaxel and then received combined 5-fluorouracil, doxorubicin, and cyclophosphamide chemotherapy. Tumor cellularity was assessed from hematoxylin and eosin-stained tissue sections as the percentage of tumor area that contained invasive carcinoma.

RESULTS. After neoadjuvant chemotherapy, tumor cellularity decreased from a median of 40% in core needle biopsy to 10% in resection specimens ($P < 0.01$; Wilcoxon signed rank test). The cellularity of core needle biopsy (median, 30%) tended to underestimate the cellularity of resection specimens (median, 40%) in the control group ($P < 0.01$). Changes in cellularity varied within each clinical response category, particularly partial response and minor response. The greatest reduction was observed in the cellularity of residual primary tumors that measured ≤ 1 cm (pathologic T1a [pT1a] and pT1b tumors), but changes in cellularity varied in the pT1, pT2, and pT3 residual tumor categories. The shape of the distribution of tumor size, expressed as the greatest dimension in cm, was similar in the control group and the treatment group (excluding complete pathologic response); however, when residual tumor size and cellularity were combined, the distribution of pathologic response shifted left (toward complete response) with a steep decline, suggesting that many tumors had a large reduction in cellularity but little change in the tumor size.

CONCLUSIONS. Cellularity of the tumor mass was reduced significantly by neoadjuvant chemotherapy, and the change varied widely in different categories of clinical response. Although residual tumors measuring ≤ 1 cm in greatest dimension had the most reduction in tumor cellularity, there was broad variability for all residual tumor groups (pT1–pT3). The frequency distribution of residual tumor size was altered markedly by the inclusion of tumor cellularity, indicating that the product of pathologic size and tumor cellularity may provide more accurate pathologic response information than tumor size alone. *Cancer* 2004;100:1365–73. © 2004 American Cancer Society.

KEYWORDS: tumor cellularity, neoadjuvant chemotherapy, clinical response, tumor size.

The response of primary breast carcinoma to neoadjuvant chemotherapy correlates with survival. Patients who achieve a complete pathologic response are reported to have significantly improved disease free and overall survival.¹⁻⁵ Patients with smaller primary tumors are more likely to achieve a complete pathologic response.⁶ The frequency of complete pathologic response (3-30%) depends on the clinical tumor classification and the type of chemotherapy used.^{1,2,7} However, 60-80% of patients achieve partial or minor responses, and their prognosis is variable; therefore, further refinement of response assessment would be informative for this predominant group.^{6,8-12}

Histologic evidence of response to preoperative chemotherapy was investigated previously in bone pathology, in which it was found that the percent tumor necrosis was the most significant prognostic factor in patients with osteosarcoma.¹³ Recently, it was demonstrated that categories of histologic change independently were predictive of 5-year survival in patients with breast carcinoma after multimodality therapy.¹⁴ We hypothesize that measurement of tumor cellularity, defined as the percentage of invasive tumor comprised of tumor cells, represents a potentially informative histologic measure of the differential response of primary breast tumors to chemotherapy. The objective of this study was to determine whether there

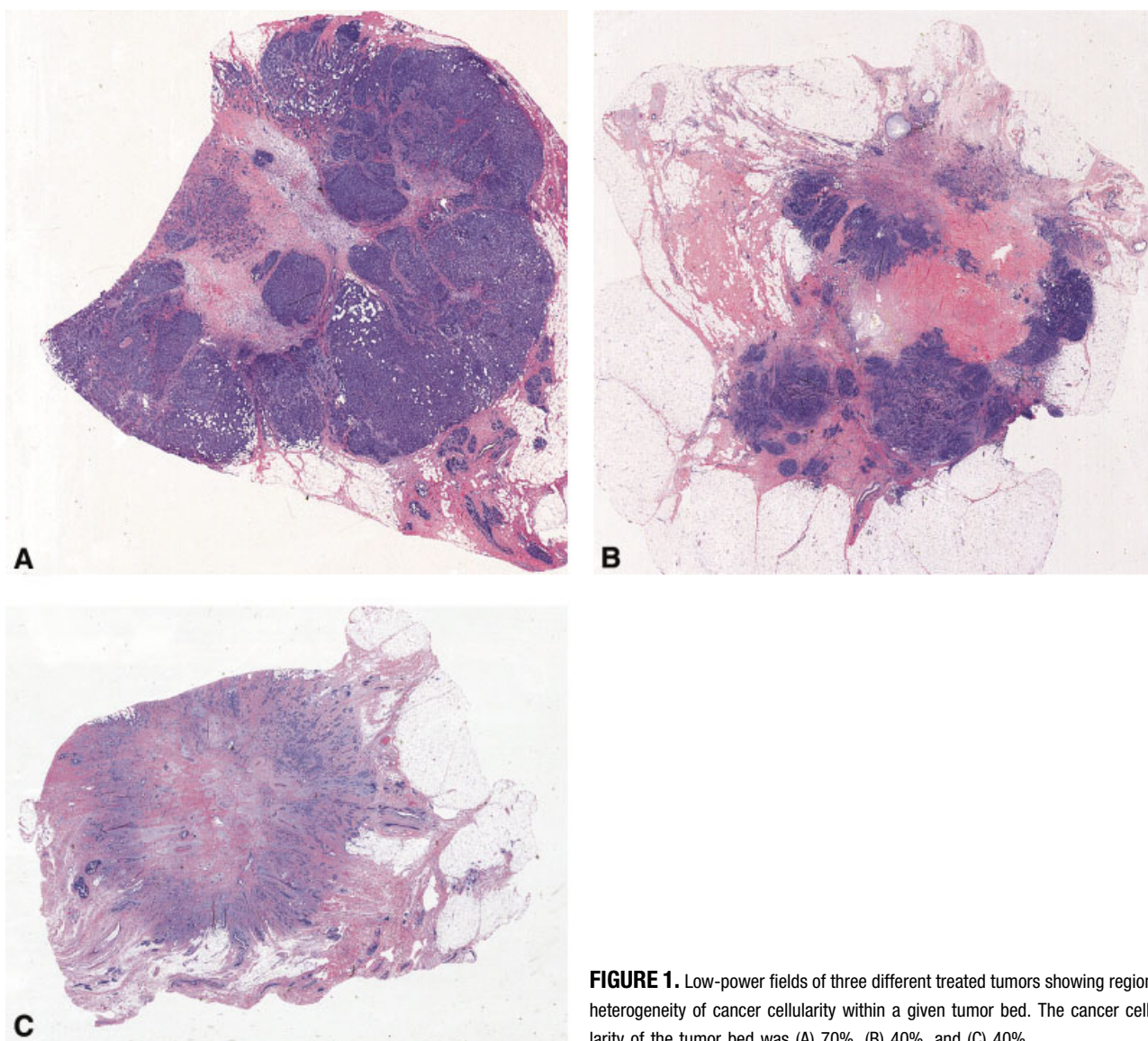


FIGURE 1. Low-power fields of three different treated tumors showing regional heterogeneity of cancer cellularity within a given tumor bed. The cancer cellularity of the tumor bed was (A) 70%, (B) 40%, and (C) 40%.

TABLE 1
Statistics of Cellularity (%) and Pathologic Tumor Size

Group	Cellularity (%)		Tumor size (cm)
	Core biopsy	Resected specimens	
Control group (<i>n</i> = 120 patients)			
Median	30	40	1.5
Range	75 (5–80)	80 (10–90)	12.8 (0.2–13.0)
Mean ± SD	38 ± 20	44 ± 20	2.1 ± 1.9
Treatment group (<i>n</i> = 120 patients)			
Median	40	10	1.3
Range	80 (10–9)	80 (0–8)	9.0 (0.0–9.0)
Mean ± SD	42 ± 21	18 ± 21	1.7 ± 1.7

SD: standard deviation.

are changes in tumor cellularity after chemotherapy, to ascertain whether there is variation in the extent of change in the different clinical response categories and residual tumor classifications, and what (if any) impact the inclusion of tumor cellularity may have on the distribution of pathologic tumor size.

MATERIALS AND METHODS

Patient Population

The patient population consisted of 240 patients with invasive breast carcinoma. The treated group was comprised of 120 patients who received neoadjuvant chemotherapy at the University of Texas M.D. Anderson Cancer Center between December 1998 and April 2001. Most treated patients (108 of 120 patients) were entered onto a clinical trial (ID 98-240) and were randomized to receive either weekly paclitaxel (150 mg/m² over 16 weeks for lymph node positive disease and 80 mg/m² over 12 weeks for lymph node negative disease) or paclitaxel given at 21-day intervals (225 mg/m²) for 4 cycles. After completion of paclitaxel, all patients received 4 additional cycles of 5-fluorouracil (500 mg/m²), doxorubicin (50 mg/m²), and cyclophosphamide (500 mg/m²) (T/FAC) before surgery. The control group was comprised of 120 patients who were treated by primary surgical management up to 4 weeks after core needle biopsy. All patients underwent core needle biopsy (14-gauge or 18-gauge) of the tumor for initial diagnosis followed by surgical resection, either as primary management (control group) or after neoadjuvant chemotherapy (treated group). Inclusion in this study required the availability of hematoxylin and eosin-stained histologic sections both from the initial core needle biopsy and from the subsequent resection specimen. Pathologic review and data analysis were conducted in accordance with an Institutional Review Board protocol that was ap-

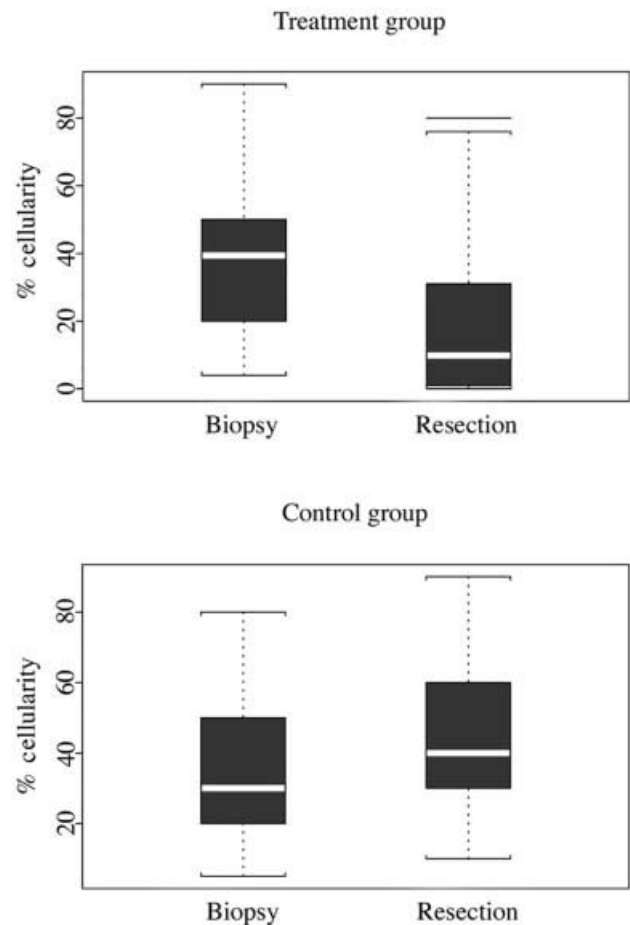


FIGURE 2. A box plot of the tumor cellularity in the core needle biopsies and resection specimens from the control group and the treatment group shows that a significant decrease in cellularity occurred within the treatment group ($P < 0.01$). The colored rectangle indicates the 25th and 75th percentiles of each distribution with the median indicated by the white horizontal line within the rectangle. The outer boundary brackets delineate the 2.5th and 97.5th percentiles, and single black lines represent individual patients outside of this range.

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Assessment of Cellularity Within the Tumor

Sections of the tumor cross-sectional area were reconstructed from 1) mapping the tissue section code from the report to the macroscopic description of the tumor bed, 2) known macroscopic tumor dimensions from the report, and 3) comparison with available specimen radiographs. The boundaries of the tumor area were then outlined on the slides with ink. Computer-generated images of known areas were created in 10% increments to simulate different microscopic patterns of cancer and were used for initial visual training.

TABLE 2

Categorization of the Change in Tumor Cellularity, Tumor Size (cm), and Tumor Size Multiplied by Cellularity according to Clinical Response and Residual Tumor Stage^a

Variable	No.	Change in tumor cellularity			Tumor size (cm)		Tumor size × cellularity		
		Median	Range	Mean ± SD	Median	Mean ± SD	Median	Range	Mean ± SD
Response									
CR	31	−1.0	0.95 (−1.0, −0.05)	−0.85 ± 0.3	0.0	0.8 ± 1.6	0.0	1.2 (0.0, 1.2)	0.09 ± 0.24
PR	62	−0.6	3.2 (−1.0, 2.2)	−0.3 ± 0.7	1.4	1.5 ± 1.4	0.2	4.5 (0.0, 4.5)	0.38 ± 0.62
MR	12	−0.5	2.48 (−0.98, 1.5)	−0.1 ± 0.9	2.1	2.7 ± 2.1	0.8	1.4 (0.0, 1.4)	0.7 ± 0.4
PD	3	−0.05	0.16 (−0.2, −0.04)	−0.1 ± 0.09	1.3	3.9 ± 4.4	0.98	3.35 (0.25, 3.6)	1.6 ± 1.8
Tumor stage									
T0	28	−1.0	0.0 (−1.0, −1.0)	−1.0 ± 0.0	0.0	0.0 ± 0.0	0.0	0.0 (0.0, 0.0)	0.0 ± 0.0
T1a	7	−0.6	1.9 (−0.98, 1.0)	−0.3 ± 0.8	0.4	0.3 ± 0.17	0.1	0.15 (0.001, 0.15)	0.08 ± 0.07
T1b	15	−0.8	2.3 (−0.98, 1.3)	−0.5 ± 0.7	0.9	0.8 ± 0.16	0.09	0.45 (0.009, 0.46)	0.1 ± 0.1
T1c	34	−0.3	3.1 (−0.97, 2.2)	−0.2 ± 0.7	1.5	1.5 ± 0.25	0.3	1.2 (0.0, 1.2)	0.4 ± 0.4
T2	17	−0.4	2.7 (−0.85, 1.9)	−0.07 ± 0.7	2.5	2.9 ± 0.8	0.5	1.3 (0.12, 1.4)	0.6 ± 0.4
T3	7	−0.7	0.9 (−0.85, 0.0)	−0.5 ± 0.4	6.5	6.9 ± 1.3	0.8	4.25 (0.25, 4.5)	1.6 ± 1.7

SD: standard deviation; CR: complete response; PR: partial response; MR: minor response; PD: progressive disease.

^a Change in cellularity was defined as (% cellularity of resection - % cellularity of core needle biopsy) / % cellularity of core needle biopsy.

Cellularity within the tumor area was assessed from the slides by estimating the percentage area of the overall tumor bed that was comprised of invasive tumor cells. The complete cross-sectional area of the tumor bed was studied to account for the heterogeneous distribution of tumor cells within a given tumor bed (Fig. 1). Three pathologists independently reviewed the percentage tumor cellularity in the first 70 specimens, and there was nearly complete concordance between pathologists. One pathologist then completed the analysis. In specimens with multifocal disease, cellularity was assessed in the same tumor mass that had been sampled by core needle biopsy. Cellularity was recorded in 10% increments from 10% to 100%, with additional values of 1% and 5% for minimal cellularity. The proportion of invasive carcinoma was then calculated.

Clinical Response Categories

The assessment of clinical response was based on change in tumor size from pretreatment clinical measurements to posttreatment clinical and radiologic measurements. The clinical measurement was the product of the two greatest palpable perpendicular dimensions of the tumor. Clinical response was categorized into four groups: a complete response (CR) was defined as complete resolution of all tumor determined by physical examination and imaging studies; a partial response (PR) was defined as incomplete reduction > 50% in tumor size; a minor response (MR) was defined as a reduction in tumor size < 50%; and progressive disease (PD) was defined as an increase in

tumor size. Pathologic size was defined as the greatest dimension of residual invasive tumor and was categorized using the revised American Joint Committee on Cancer TNM staging system.¹⁵

Statistical Analyses

Distributions of cellularity percentages among groups are summarized graphically using box plots. The shaded rectangles in the box plots delineate the 25th and 75th percentiles of each distribution, with the median indicated by a horizontal white line within the rectangle. The outer boundary brackets delineate the 2.5th and 97.5th percentiles. Black lines then represent individual results outside of this range. The distributions of 1) residual pathologic tumor size and 2) the product of residual pathologic tumor size and tumor cellularity are summarized graphically by histograms. Measurements of cellularity in core needle biopsy and resection specimens were compared using the Wilcoxon signed rank test. All *P* values presented are two-sided, and *P* values < 0.05 were considered statistically significant. Statistical analyses were performed using SAS software (version 8.0) and Splus software (version 6.0). The relative change in tumor cellularity was computed with the following formula: relative change in tumor cellularity = (percentage tumor cellularity at resection - percentage tumor cellularity in the core needle biopsy) / percentage tumor cellularity in the core needle biopsy. Negative values indicated lower cellularity at resection compared with the core needle biopsy specimen. A minimum value of -1.0 was equated with a CR.

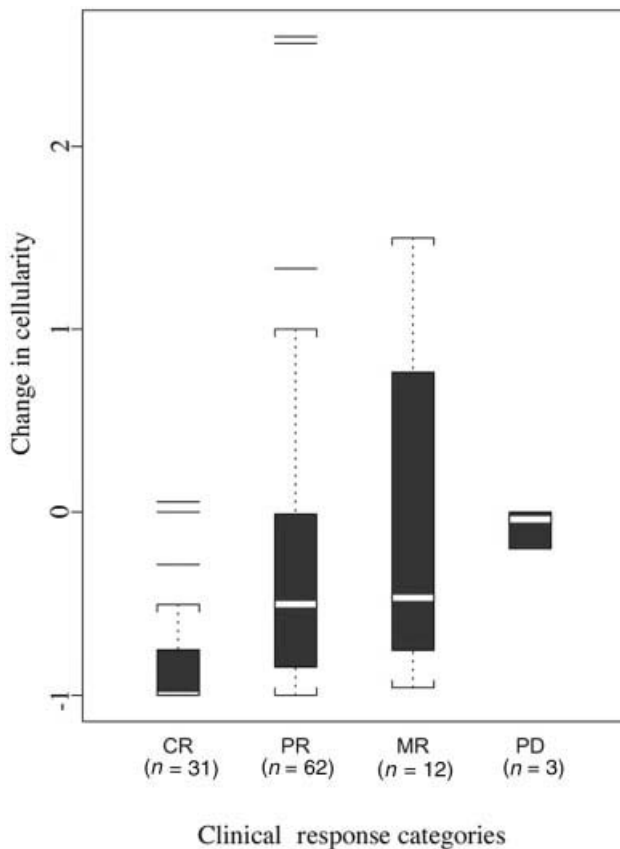


FIGURE 3. Relative change in tumor cellularity for each clinical response category: complete response (CR), partial response (PR), minor response (MR), and progressive disease (PD). Relative change in tumor cellularity was calculated as follows: (percentage tumor cellularity at resection – percentage tumor cellularity in core needle biopsy)/percentage tumor cellularity in core needle biopsy. The colored rectangle indicates the 25th and 75th percentiles of the distribution, and the median is indicated by the white horizontal line within the rectangle. The outer boundary brackets delineate the 2.5th and 97.5th percentiles, and short horizontal lines represent individual patients outside of this range.

RESULTS

Statistics of the percentage tumor cellularities are presented for all groups (Table 1). Within the treated group, the median tumor cellularity decreased significantly from 40% in the core needle biopsies to 10% in the resected tumors ($P < 0.01$; Wilcoxon signed rank test). Tumor cellularity in patients from the control group increased from a median of 30% (core needle biopsy) to 40% (resected tumor; $P < 0.01$), indicating that core needle biopsy specimens may underestimate the overall cellularity at resection. These data are summarized in Figure 2 using a box plot.

Clinical response data were available for the 108 patients who received T/FAC neoadjuvant chemotherapy. The response rates in the current series (29%

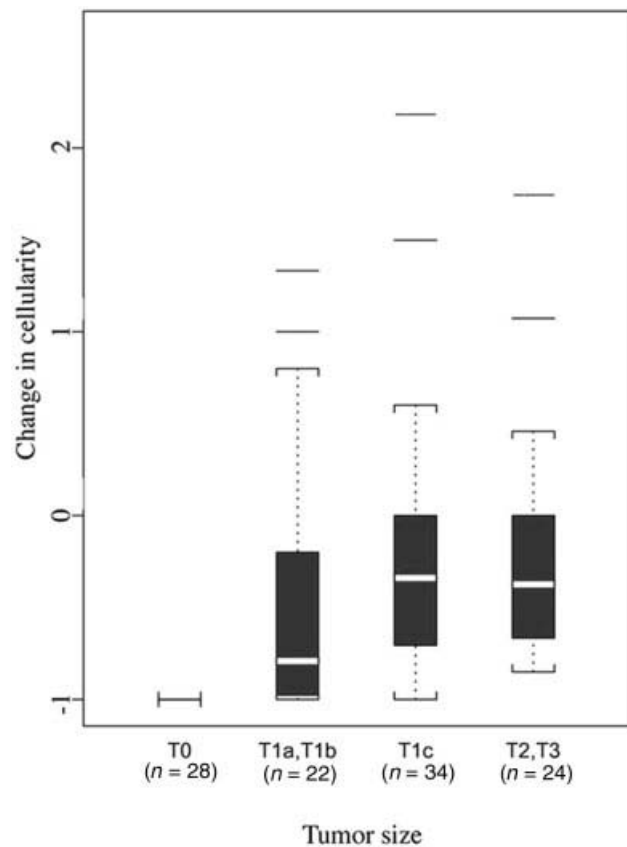


FIGURE 4. Relative changes in cellularity categorized by tumor stage show that T1a and T1b residual tumors demonstrate the greatest change in cellularity. However, changes in cellularity were found to be highly variable in T1, T2, and T3 residual tumors. Tumor size was categorized using the revised American Joint Committee on Cancer TNM staging system. This box plot format is the same as that used in Figure 3.

clinical CR, 57% PR, 11% stable disease, and 3% PD) are in agreement with those reported in most studies^{1–12} and for this clinical trial.⁷ The change in tumor cellularity relative to the starting value in the core needle biopsy was compared with clinical response and residual pathologic tumor (pT) status (Table 2). Relative changes in cellularity were highly variable in all four clinical response groups, particularly for patients who achieved a PR or an MR (Table 2, Fig. 3). Change in cellularity is related to clinical response: There was a median 50% reduction in tumor cellularity in the PR and MR groups (Table 2), although some tumors had increased cellularity, and the few tumors that progressed had no median change in tumor cellularity (Fig. 3). Categorization by residual pathologic tumor status shows that changes in cellularity were highly variable for all residual tumor classifications (pT1–pT3), but that pT1a and pT1b tumors (combined) showed the greatest reduction in cellularity

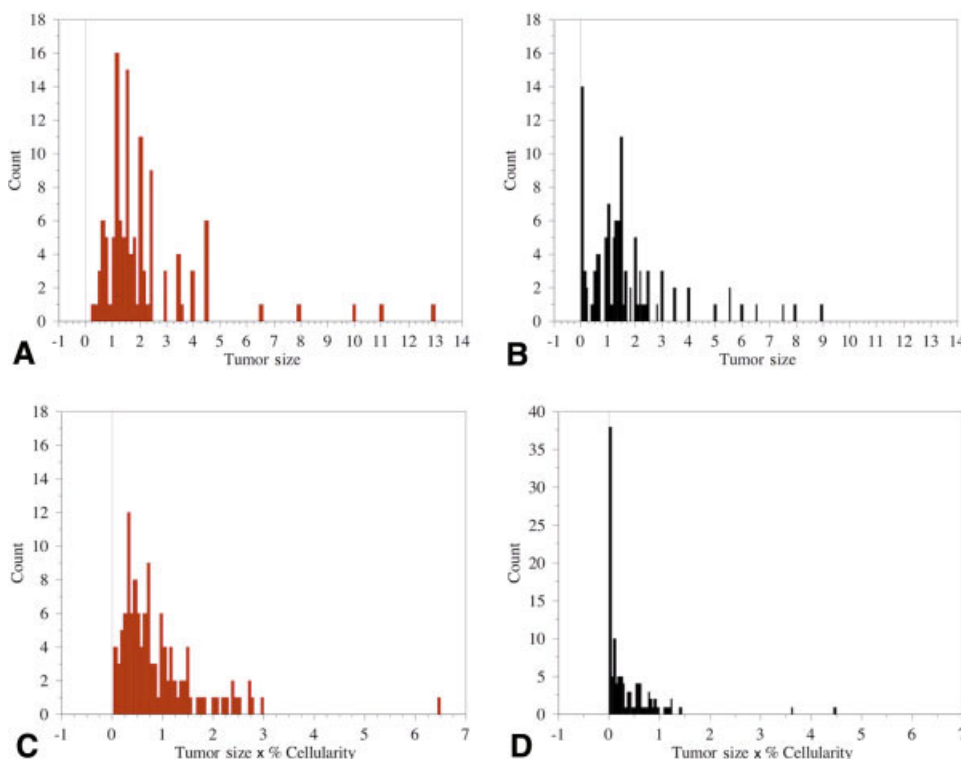


FIGURE 5. Histograms of pathologic tumor size (in cm) for the (A) control group and (B) treatment group show that the shapes of these distributions are similar (excluding the peak of complete pathologic responses in the treatment group). Histograms of the product of cancer cellularity and tumor size for the (C) control group and (D) treatment group show that the shape of the distribution for the control group is similar to that for tumor size (A). However, in treated tumors, the shape of the distribution changes to become a steeply inversely sloping curve. The control group is shown in red (A and C), and the treated group is shown in black (B and D).

(Fig. 4). Residual pT1a and pT1b tumors had similar median reductions in cellularity. A minority of tumors in each residual tumor classification had increased cellularity after treatment (see Fig. 4, positive values).

The frequency distributions of pathologic tumor size alone have similar shapes in the control group (Fig. 5A) and the treatment group when pathologic CRs are excluded in the treatment group (Fig. 5B). However, the product of pathologic size and tumor cellularity produces a steeply inversely sloped distribution in the treatment group (Fig. 5D) whereas the shape of the distribution in control group is similar to the distribution for size alone (Fig. 5C). The product of cellularity and size dramatically changes the distribution of residual tumor pathology in the treated group, causing a shift toward CR. This indicates that chemotherapy in some tumors can reduce cellularity dramatically but affects the overall size of the tumor only minimally. We propose that the product of residual size and cellularity may be a more clinically relevant measure of tumor response than assessing tumor size alone.

DISCUSSION

Clinical trials consistently indicate that the extent of response of primary breast carcinoma to neoadjuvant chemotherapy correlates with disease-free and overall survival.¹⁻¹² The currently used categories of clinical

response (namely, CR, PR, MR, and PD) are defined by the change in tumor size from pretreatment clinical and/or radiologic measurements to posttreatment clinical, radiologic, and pathologic measurements. However, residual tumor size is influenced by variable pathologic changes that occur within the tumor bed. Chemotherapy-induced fibrous stromal involution is reported to occur in up to 67% of tumors¹⁶ and can result in clinical and macroscopic overestimation of residual tumor size. There is clearly a role for the development and validation of new histologic approaches to augment the pathologic and clinical assessment and to provide information concerning the differential response to neoadjuvant chemotherapy, particularly for tumors that achieve less than a pathologic CR.

The current study assessed the role that microscopic assessment of tumor cellularity may have in the pathologist's evaluation of tumor response. There is precedent for using microscopic assessments of the percentage tumor area or cellularity in breast pathology, such as in the assessment of the amount of intraductal component of tumor sections and in the assessment of estrogen receptor, progesterone receptor, Her-2/*neu*, and proliferation index (Ki-67) immunostaining.^{17,18} In the field of bone pathology, it has been shown that histopathologic measurement of the percent tumor necrosis is the most significant prog-

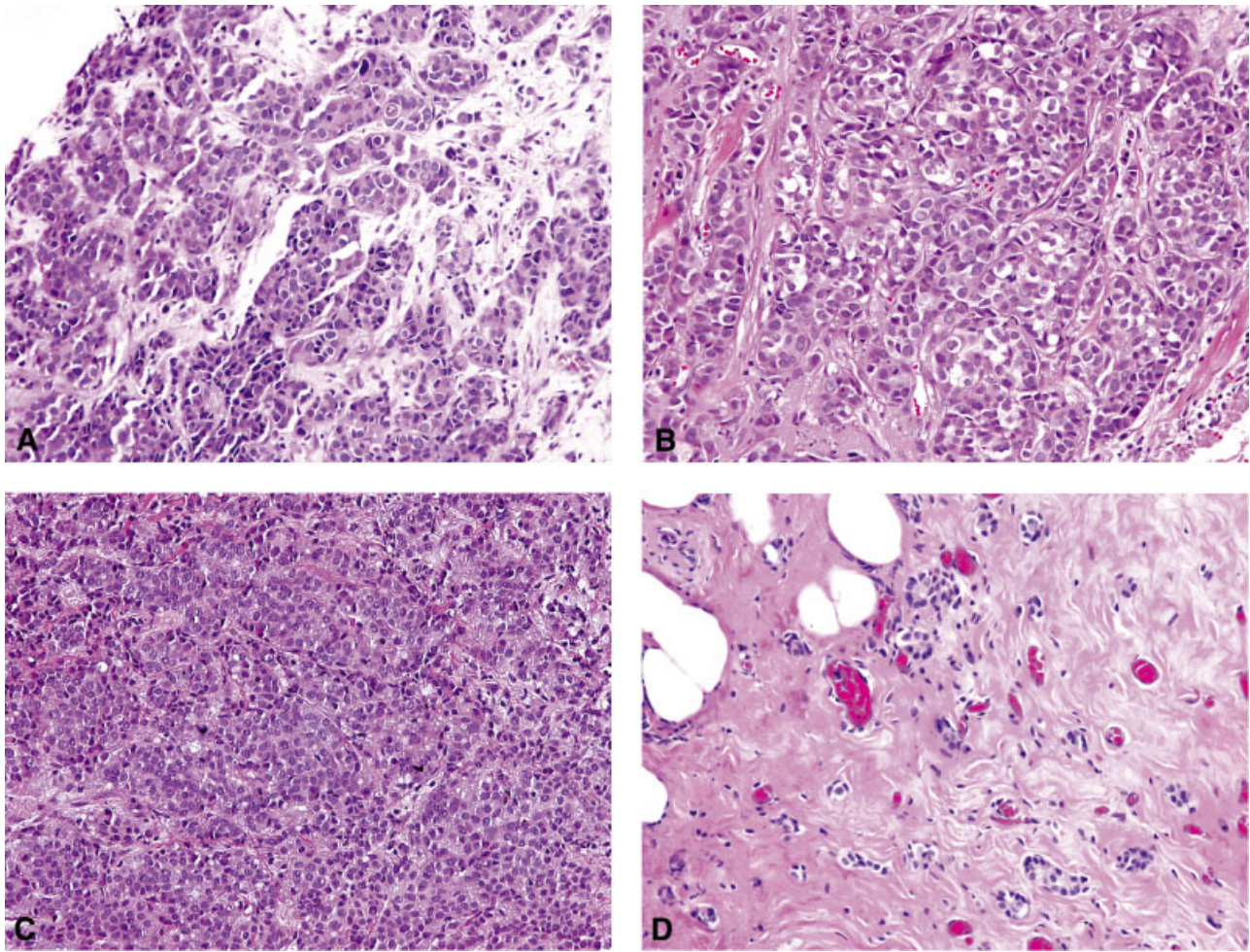


FIGURE 6. Two partially responding tumors with a similar decrease in tumor size but with markedly different changes in cellularity after neoadjuvant chemotherapy. (A, B) One tumor decreased from 2.0 cm to 1.8 cm and showed an increase in cellularity. A pretreatment core needle biopsy with a cellularity of 70% is shown in A, and a posttreatment tumor with a cellularity of 80% is shown in B. (C, D) The second tumor decreased from 1.7 cm to 1.5 cm and showed a decrease in cellularity. A pretreatment core needle biopsy with a cellularity of 90% is shown in C, and a posttreatment tumor with a cellularity of 5% is shown in D (original magnification $\times 10$).

nostic factor in patients with osteosarcoma who are treated with preoperative chemotherapy.¹³ We defined the size of the residual tumor bed; then, we estimated the overall cellularity of invasive tumor within that tumor bed. A potential benefit of this approach in the pathologic assessment after chemotherapy is that it bypasses the difficulties in measuring the greatest dimension of invasive tumor that is distributed unevenly within the residual tumor bed as scattered islands of residual disease.

The current results showed that the cellularity of the tumor mass is reduced significantly by neoadjuvant chemotherapy and that change is widely variable between individual patients and in the different categories of clinical response and residual tumor sizes. Relative change in cellularity varies widely in tumors

that achieve a PR of MR and in the different residual tumor classifications. Figure 6 illustrates two partially responsive tumors that had similar decreases in tumor size after chemotherapy yet showed markedly different changes in cellularity. Changes in tumor size alone do not represent response entirely. Tumor cellularity in patients from the control group increased from a median of 30% to 40% ($P < 0.01$), indicating that core needle biopsy may underestimate the overall cellularity at resection. Preferential sampling by core needle biopsy of the fibrotic center in that subset of tumors may lower the median.¹⁹ It is possible that artifactual tissue crushing from the automated core needle biopsy device may compress the cellular component more than the intervening stroma, hence slightly decreasing apparent cellularity. There may be differ-

ences in cell areas from different fixation and processing schedules for core needle biopsy and resection specimens. Uninhibited growth of tumor is unlikely to be a contributing factor, because resection shortly followed biopsy.

The current study is too recent to generate survival data. However, a recent retrospective study of 176 patients used broad categories of reduction in tumor cellularity and demonstrated a significant correlation with overall and disease free survival at 5 years.¹⁴ In that study, the authors analyzed the histologic response as an independent variable and did not compare this with clinical response or residual pathologic tumor size.¹⁴ That study adds support to our finding that the change in cellularity within the tumor is an independent variable to be included in the pathologic assessment and to be combined with change in the tumor size.

When pathologic CRs (pT0) were excluded, the distribution of pathologic size in treated and control tumors appeared to have similar shapes, distributed asymmetrically around a modal peak. That shape of distribution in the treated group may be interpreted to mean that tumors that do not achieve a pathologic CR are not affected much as a population (Fig. 5B). However, the shape of the distribution of the product of tumor size and tumor cellularity demonstrated a marked left shift in the population to form an inversely sloping curve (Fig. 5D). That distribution suggests that many tumors nearly achieve a pathologic CR. Indeed, the variable cellularity of residual tumors appears to organize the pathologic responses when it is included with residual tumor size (Fig. 5D). Other clinical studies have indicated that smaller residual tumors, such as microscopic residual invasive carcinoma, and even tumors that measure < 1 cm in greatest dimension, are associated with improved survival compared with other residual tumors.^{6,16,20} The observed reduction in cellularity in pT1a and pT1b tumors helps to explain this. The histogram we observed for the product of tumor size and cellularity may describe a continuous distribution of pathologic responses and may be more accurate than stratification into categories of response based on tumor size alone.

Studies currently are underway to measure the product of tumor size and cellularity of each tumor before treatment to compare with residual pathologic findings after treatment. It even may be possible to develop a mathematical model from these distributions to compare the responses of entire populations of patients who receive different neoadjuvant chemotherapy regimens. We conclude that tumor cellularity qualifies as an informative parameter for inclusion in a schema to quantify response of breast carcinoma to neoadjuvant chemotherapy.

REFERENCES

1. Bonadonna G, Valagussa, Brambilla C, et al. Primary chemotherapy in operable breast cancer: eight-year experience at the Milan Cancer Institute. *J Clin Oncol*. 1998;16:93-100.
2. Bryant J, Fisher B, Wolmark N, et al. Effect of preoperative chemotherapy on the outcome of women with operable breast cancer. *J Clin Oncol*. 1998;16:2672-2685.
3. Ferriere JP, Assier I, Cure H, et al. Primary chemotherapy in breast cancer: correlation between tumor response and patient outcome. *Am J Clin Oncol*. 1998;21:117-120.
4. Honkoop AH, van Diest PJ, de Jong JS, et al. Prognostic role of clinical, pathological and biological characteristics in patients with locally advanced breast cancer. *Br J Cancer*. 1998;77:621-626.
5. Lenert JT, Vlastos G, Mirza NQ, et al. Primary tumor response to induction chemotherapy as a predictor of histological status of axillary nodes in operable breast cancer patients. *Ann Surg Oncol*. 1999;6:762-767.
6. Kuerer HM, Newman LA, Smith TL, et al. Clinical course of breast cancer patients with complete pathologic primary and axillary lymph node response to doxorubicin-based neoadjuvant chemotherapy. *J Clin Oncol*. 1999;17:460-467.
7. Green MC, Buzdar AU, Smith T, et al. Weekly paclitaxel followed by FAC as primary systemic chemotherapy of operable breast cancer improves pathologic complete remission (pCR) rates when compared to every 3-week therapy followed by FAC-final results of a prospective Phase III randomized trial. *Proc Am Soc Clin Oncol*. 2002;21:135.
8. Chollet P, Amat S, Cure H, et al. Prognostic significance of a complete pathological response after induction chemotherapy in operable breast cancer. *Br J Cancer*. 2002;86:1041-1046.
9. Kuerer HM, Newman LA, Buzdar AU, et al. Pathologic tumor response in the breast following neoadjuvant chemotherapy predicts axillary lymph node status. *Cancer J Sci Am*. 1998;4:230-236.
10. Valero V, Buzdar AU, McNeese M, Singletary E, Hortobagyi GN. Primary chemotherapy in the treatment of breast cancer: the University of Texas M. D. Anderson Cancer Center experience. *Clin Breast Cancer*. 2002;3(Suppl 2):S63-S68.
11. Wolmark N, Wang J, Mamounas E, Bryant J, Fisher B. Preoperative chemotherapy in patients with operable breast cancer: nine-year results from the National Surgical Adjuvant Breast and Bowel Project. *J Natl Cancer Inst Monogr*. 2001;30:96-102.
12. Machiavelli MR, Romero AO, Perez JE, et al. Prognostic significance of pathological response of primary tumor and metastatic axillary lymph nodes after neoadjuvant chemotherapy for locally advanced breast carcinoma. *Cancer J Sci Am*. 1998;4:125-131.
13. Raymond AK, Chawla SP, Carrasco H, et al. Osteosarcoma Chemotherapy effect: a prognostic factor. *Semin Diagn Pathol*. 1987;4:212-236.
14. Ogston KN, Miller ID, Payne S, et al. A new histological grading system to assess response of breast cancers to primary chemotherapy: prognostic significance and survival. *Breast*. 2003;12:320-327.
15. Singletary SE, Allred C, Ashley P, et al. Revision of the American Joint Committee on Cancer staging system for breast cancer. *J Clin Oncol*. 2002;20:3576-3577.

16. Fisher ER, Wang J, Bryant J, Fisher B, Mamounas E, Wolmark N. Pathobiology of preoperative chemotherapy. Findings from the National Surgical Adjuvant Breast and Bowel Project (NSABP) Protocol B-18. *Cancer*. 2002;95:681–695.
17. Furukawa Y, Kimijima II, Abe R. Immunohistochemical image analysis of estrogen and progesterone receptors in breast cancer. *Breast Cancer*. 1998;5:375–380.
18. Auger M, Katz RL, Johnston DA, Sneige N, Ordonez NG, Fritsche H. Quantitation of immunocytochemical estrogen and progesterone receptor content in fine needle aspirates of breast carcinoma using the SAMBA 4000 image analysis system. *Anal Quant Cytol Histol*. 1993;15:274–280.
19. Morris EA, Liberman L, Trevisan SG, Abramson AF, Dershaw DD. Histologic heterogeneity of masses at percutaneous breast biopsy. *Breast J*. 2002;8:187–191.
20. Mauriac L, MacGrogan G, Avril A, et al. Neoadjuvant chemotherapy for operable breast carcinoma larger than 3 cm: a unicentre randomized trial with a 124-month median follow-up. Institut Bergonie Bordeaux Groupe Sein (IBBGS). *Ann Oncol*. 1999;10:47–52.